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<110> SAHIN, ERINC
    TARALP, ALPAY
    SAYERS, SEHRA

<120> CIRCULAR RECOMBINANT PLASMID DNA CONSTRUCTS AND THEIR PROTEIN PRODUCTS, METHODS OF PREPARATION AND IMMOBILISATION OF PROTEINS ON SUPPORT

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<141> 2005-09-20

<150> PCT/TR2003/000019
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ggt gat gtt aat ggg cac aaa ttt tct gtc agt gga gag ggt gaa ggt   393
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly
           25   30   35

gat gca aca tac gga aaa ctt acc ctt aaa ttt att tgc act act gga   441
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly
      40   45   50

aaa cta cct gtt cca tgg cca aca ctt gtc act act ttc tct tat ggt   489
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly
       55   60   65

gtt caa tgc ttt tcc cgt tat ccg gat cat atg aaa cgg cat gac ttt   537
Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg His Asp Phe
    70   75   80

ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa cgc act ata tct   585
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Ser
85   90   95   100

ttc aaa gat gac ggg aac tac aag acg cgt gct gaa gtc aag ttt gaa   633
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
        105   110   115

ggt gat acc ctt gtt aat cgt atc gag tta aaa ggt att gat ttt aaa   681
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys
       120   125   130

gaa gat gga aac att ctc gga cac aaa ctc gag tac aac tat aac tca   729
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser
     135   140   145

cac aat gta tac atc acg gca gac aaa caa aag aat gga atc aaa gct   777
His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala
   150   155   160

aac ttc aaa att cgc cac aac att gaa gat gga tcc gtt caa cta gca   825
Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala
165   170   175   180

gac cat tat caa caa aat act cca att ggc gat ggc cct gtc ctt tta   873
Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu
      185   190   195

cca gac aac cat tac ctg tcg aca caa tct gcc ctt tcg aaa gat ccc   921
Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro
     200   205   210

aac gaa aag cgt gac cac atg gtc ctt ctt gag ttt gta act gct gct   969
Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala
    215   220   225

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Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys   Ile Pro
    230   235   240

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Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35   40   45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50   55   60

Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65   70   75   80

Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85   90   95

Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100   105   110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115   120   125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130   135   140

Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
145   150   155   160

Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
        165   170   175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
      180   185   190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
     195   200   205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
  210   215   220

Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225   230   235

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1   5   10   15

act act gag aat ctt tat ttt cag ggc gcc atg gga ggc acg gta ccg   96
Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Gly Thr Val Pro
      20   25   30

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gta gaa aaa atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca   144
Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
    35   40   45

att ctt gtt gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc   192
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
   50   55   60

agt gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa   240
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65   70   75   80

ttt att tgc act act gga aaa cta cct gtt cca tgg cca aca ctt gtc   288
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
      85   90   95

act act ttc tct tat ggt gtt caa tgc ttt tcc cgt tat ccg gat cat   336
Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
    100   105   110

atg aaa cgg cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta   384
Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
   115   120   125

cag gaa cgc act ata tct ttc aaa gat gac ggg aac tac aag acg cgt   432
Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
   130   135   140

gct gaa gtc aag ttt gaa ggt gat acc ctt gtt aat cgt atc gag tta   480
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
145   150   155   160

aaa ggt att gat ttt aaa gaa gat gga aac att ctc gga cac aaa ctc   528
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
    165   170   175

gag tac aac tat aac tca cac aat gta tac atc acg gca gac aaa caa   576
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
   180   185   190

aag aat gga atc aaa gct aac ttc aaa att cgc cac aac att gaa gat   624
Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
   195   200   205

Page 17

gga tcc gtt caa cta gca gac cat tat caa caa aat act cca att ggc   672
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
 210   215   220

gat ggc cct gtc ctt tta cca gac aac cat tac ctg tcg aca caa tct   720
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225   230   235   240

gcc ctt tcg aaa gat ccc aac gaa aag cgt gac cac atg gtc ctt ctt   768
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
           245   250   255

gag ttt gta act gct gct ggg att aca cat ggc atg gat gag ctc cgt   816
Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
           260   265   270

cga caa gct tgc ggc cgc act cga gca cca cca cca cca cca ctg aga   864
Arg Gln Ala Cys Gly Arg Thr Arg Ala Pro Pro Pro Pro Pro Leu Arg
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Ser Gly Cys
  290

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<220>
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        35   40   45

Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
    50   55   60

Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65   70   75   80

Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
       85   90   95

Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
     100   105   110

Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
     115   120   125

Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
   130   135   140

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
145   150   155   160

Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
     165   170   175

Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
     180   185   190

Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
     195   200   205

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
    210   215   220

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225   230   235   240

Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
     245   250   255

Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
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Ser Gly Cys
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result of plasmid modification

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<220>
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between the tag and GFP protein segments

<400> 9

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1   5

<210> 10
<211> 6
<212> PRT

<213> Artificial sequence

<220>
<223> Peptide design based on charge and shape to bind the
expressed protein to a suitably interactive surface

<400> 10

His His His His His His
1   5

<210> 11
<211> 238
<212> PRT
<213> Aequorea victora

<220>
<221> MISC_FEATURE
<223> Green fluorescent peptide coded by pGFPuv plasmid, permitting
easy visualisation and quantification based on fluorescence properties

<400> 11

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1   5   10   15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20   25   30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35   40   45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50   55   60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65   70   75   80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
       85   90   95

Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
      100   105   110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
     115   120   125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
   130   135   140

Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly
145   150   155   160

Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
      165   170   175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
     180   185   190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
     195   200   205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
   210   215   220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225   230   235

<210> 12
<211> 291
<212> PRT
<213> Artificial sequence

<220>
<223> Completed peptide based on Hisx6 tag, short physically
flexible linker and green fluorescent protein coded by
pETM-GFP-Imm to impart fluorescence properties, allowing easy
immobilisation with retention of bioactivity, visualisation
and quantification

<400> 12

Met Lys His His His His His His Pro Met Ser Asp Tyr Asp Ile Pro
1   5   10   15

Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Gly Thr Val Pro
            20   25   30

Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
        35   40   45

Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
        50   55   60

Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65   70   75   80

Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
            85   90   95

Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
            100   105   110

Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
        115   120   125

Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
        130   135   140

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
145   150   155   160

Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
        165   170   175

Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
        180   185   190

Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
        195   200   205

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
    210   215   220

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225   230   235   240

Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
        245   250   255

Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
        260   265   270

Arg Gln Ala Cys Gly Arg Thr Arg Ala Pro Pro Pro Pro Leu Arg
        275   280   285

Ser Gly Cys
    290

<210> 13
<211> 29
<212> DNA
<213> Artificial sequence

<220>
<223> Frame adapter

<400> 13

gtacgccatg ggaggcacgg taccttgtg   29